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Preliminary Communication

Chromosomal Damage in LSD Users

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An increase of chromosomal abnormalities was found in leukocytes of LSD users compared to drug-free controls. Elevated breakage rates were also found in children exposed to the drug in utero. The data showed no correlation between amount of drug per dose, number of doses, or total dosage and frequency of chromosomal breaks.

Whether chromosomal damage is inflicted by the psychotomimetic lysergic acid diethylamide (LSD) is a subject of current controversy. The findings of Cohen et al[1,2] as well as our own observations[3] have shown that pure LSD can produce in vitro and illicit LSD, possible in vivo chromosomal aberrations. However, in a study of eight cases, Loughman et al[4] reported no differences in breakage rate between LSD users and control subjects.

The present communication extends our preliminary study[3] to a significant sampling of LSD users.

Materials and Methods

Blood samples for chromosomal analysis were obtained from 50 LSD users (of whom four had been exposed to the drug in utero), and from 14 drug-free controls. All subjects were white and between 17 and 30 years of age except for the four children, one 43-year-old male user, and one 34-year-old female user.

Replicate cultures were started on commercially available microculture tubes and on media prepared according to our own technique.[5] As no significant differences were seen with the two media, the final results were pooled for analysis. After 70 hours of incubation at 37 C, demecolcine (Colcemid), 0.5μg/ml, was added for an additional two hours of incubation. Chromosomal preparations were done as described.[5] The culture tubes and slides were coded and the preparations evaluated on a blind basis. Two hundred well-spread metaphases were selected under low power (×125) from five slides per subject. Detailed analysis of the metaphases was done with oil immersion (×1,250).

Determination of breaks was based on clear discontinuity of the chromatids and displacement of the distal fragment(s) from the axis of the proximal portion. Breaks were classified as either chromatid (Fig 1), when one chromatid was affected, or isochromatid (Fig 2), when both chromatids were broken at the same point. Single fragments were classified as chromatid breaks, double fragments as isochromatid breaks (Fig 3). Gaps or discontinuity of the chromatids without displacement were not considered breaks. Dicentric chromosomes (Fig 4) and exchange figures (Fig 5) were counted as two breaks. In the last 30 cases studied, each break, when possible, was assigned to a given chromosome or group of chromosomes.

Results

Fourteen drug-free control subjects showed a breakage rate of 6% to 16.5%, with a mean of 9.03% on the basis of 2,800 metaphases analyzed (Table 1). A series of seven diagnostic roentgenograms of the head and neck regions had been taken of the subject with a breakage frequency of 16.5% one to two months before sampling.

In the user group (Table 2), the average estimated dose of LSD varied between 100μg and 1,000μg; the peak dose, between 100μg and 2,800μg; and the total dose between 150μg and 70,000μg. In 43 of 46 adults, there was an increased breakage rate above the mean control value. The percentage of breaks varied between 8% and 45%, with a mean of 18.76% on the basis of 9,140 metaphases analyzed. All four infants exposed to the drug in utero showed breakage rates above the mean control value. The percentage of breaks varied between 9.5% and 28%, with a mean of 21.5% on the basis of 800 metaphases studied. The mean breakage rate for the user group was 18.99%. This is more than twice the control mean and is highly significant (t test, $P < 0.001$).

Two of the users had taken only LSD: case III-3 (16.5% breaks) and case I-13 (28% breaks). Twenty-one of the users had taken only marihuana

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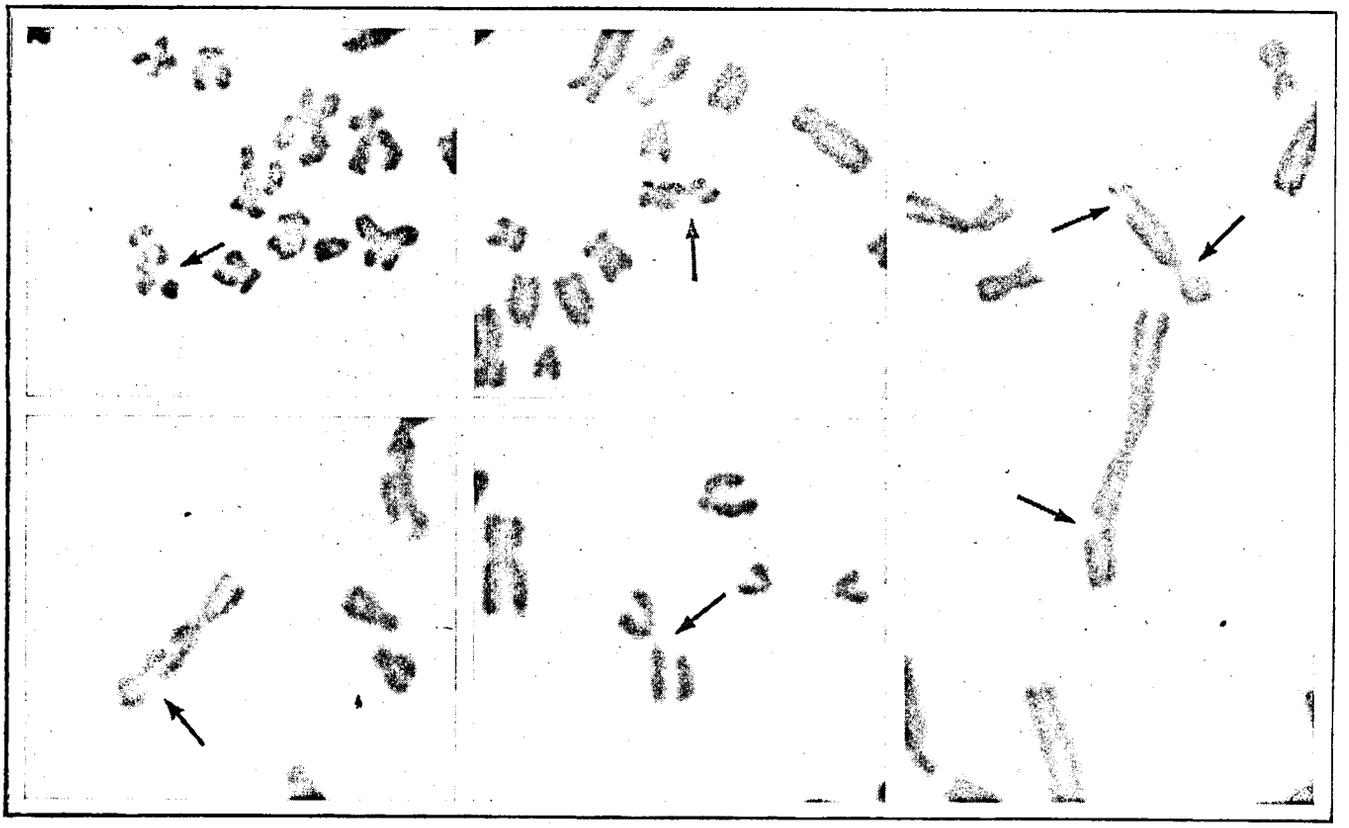


1. Chromatid breaks in persons who used LSD. Note the difference between the breaks (*arrows*) and the gaps.

in addition to LSD. The mothers of the four children studied had taken only marihuana in addition to LSD during pregnancy, but three of them had also experimented with other drugs before pregnancy occurred (Table 3). Twenty-two users had also experimented with a variety of other drugs (Table 2).

No correlation was found between the breakage rate and the number of doses, amount per dose, total dosage, or time interval between last dose and sampling; nor between the breakage rate and the number and type of other drugs used.

Most of the abnormalities were chromatid and isochromatid, but we observed 16 dicentric chromosomes and ten exchange figures. In the control group, three dicentric chromosomes and one ring chromosome were found. We also observed a total of 192 fragments that could not be assigned to a given chromosome or group of chromosomes.

Among the 50 cases studied were four children born to mothers who had ingested illicit LSD during pregnancy (Table 3); three of the children showed a very high increase in frequency of chromosomal breaks (22% to 28%). The only child with a low breakage rate (9.5%) had been exposed to a single dose of 150μg during the first month of pregnancy. In one case, the breaks were found 18 months after birth.

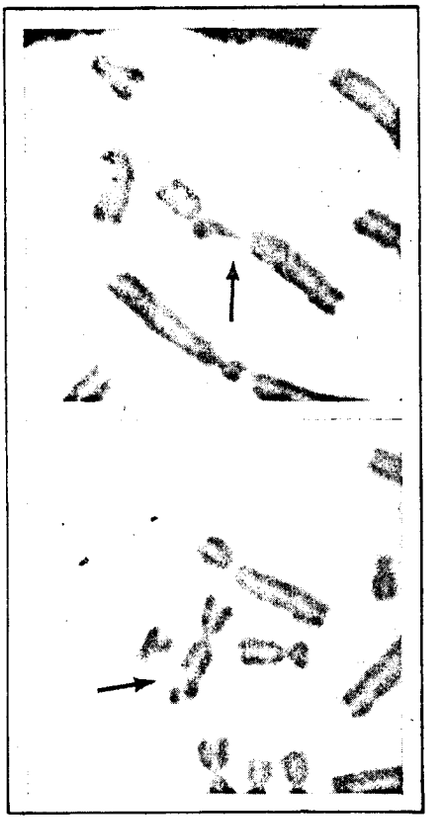
In seven cases, 0.5% to 1.5% of the cells showed only three G-group chromosomes, plus a centric fragment resembling the Philadelphia (Ph^1) chromosome of chronic myelogenous leukemia (Fig 6); the cells were otherwise normal. This abnormality was present in all children with an increased breakage rate.

Table 1.—Chromosomal Breakage in Control Group

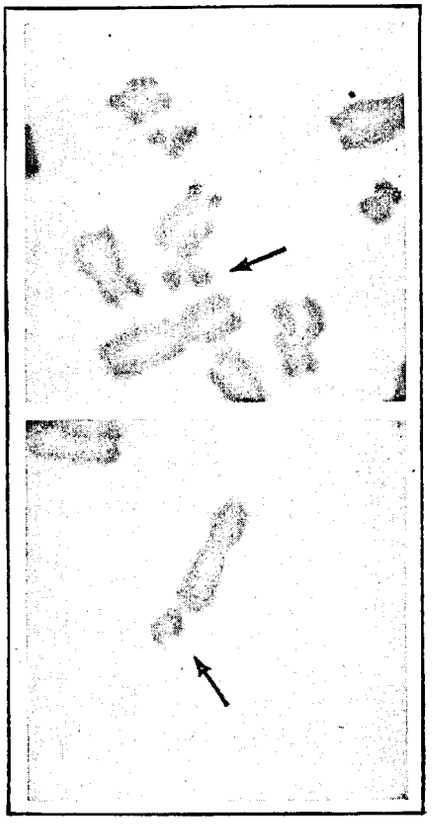
Case No.	Sex	% Breaks
I-23	M	6.0
II-94	F	6.0
I-12	F	7.0
I-14	M	7.0
II-95	F	7.5
I-24	F	7.5
I-4	M	8.5
II-85	M	8.5
I-25	F	8.5
I-16	M	9.5
I-11	F	10.0
I-3	M	10.5
I-8	M	13.5
I-6	F	16.5*

*X-ray films of this patient had recently been taken for diagnostic purposes.

In the group of 30 subjects in which breaks were assigned to a given chromosome or group of chromosomes, the distribution of chromosome breaks in the various chromosomes or groups of chromosomes was not random according to length (Table 4). Using the mean length values described in the Denver and London conferences,[6] we demonstrated a significant deviation from random in the distribution ($P < 0.001$). The reason is an excess of breaks in chromosomes 1 and 2 and a paucity of breaks in groups E and F. This distribution is somewhat different from that found by Cohen et al.[2]



2. Chromosome breaks. Both chromatids are broken at or near the same point.



3. Double fragments, resulting from isochromatid breaks.

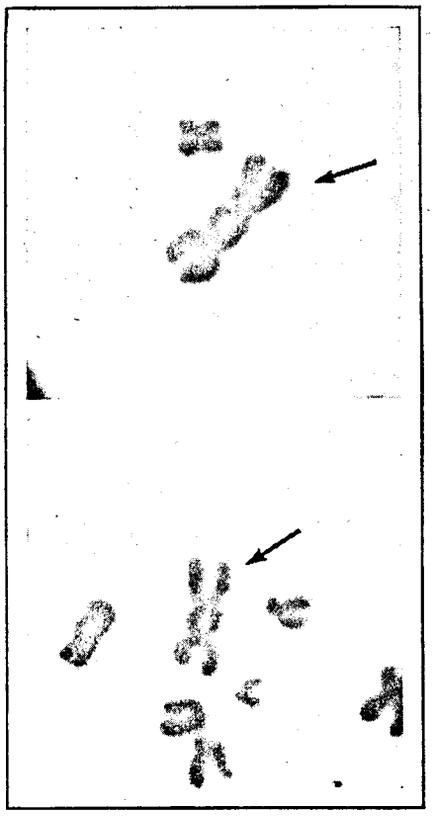
Comment

The possibility that agents commonly available in the environment can cause genetic damage is most pertinent at a moment when this environment is becoming increasingly complex. This potential danger has now been aggravated by the indiscriminate use of drugs about which little is known by a large segment of the population.

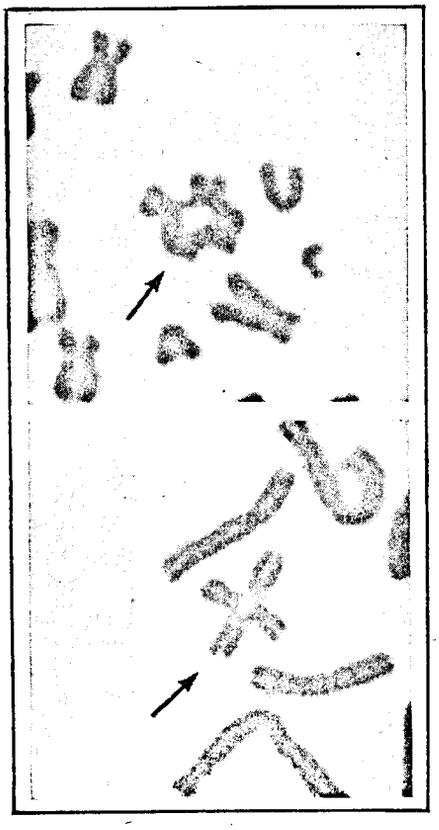
It seems probable that illicit supplies of LSD can cause chromosomal damage in the circulating blood cells of the user.[1-3,7] That LSD may be the causal agent, however, as noted by Cohen et al,[2] is suggested by the close correspondence between the frequency of breaks observed in vivo and those produced in vitro with dosages of 0.0001μg to 0.1μg/ml of LSD (doses that correspond to those the cells are exposed to in vivo).

In our study, we did not observe a depression of mitosis. This is in agreement with the in vivo findings of Cohen et al.[2] The depression of mitosis in vitro can

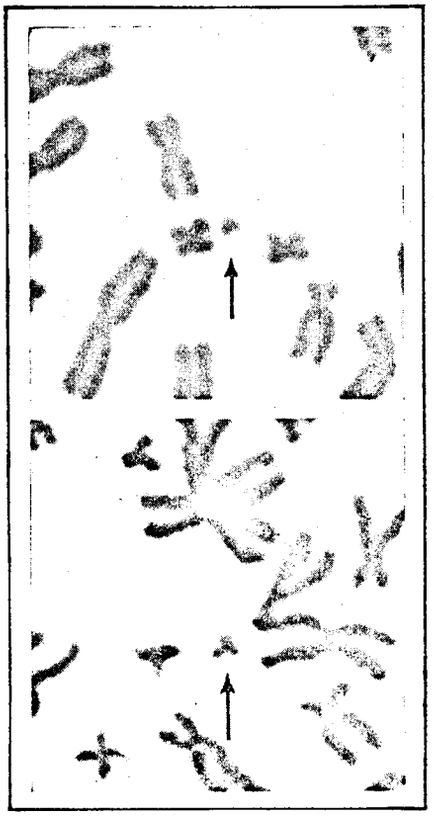
4. Dicentric chromosomes.



5. Two different types of exchange figures.



6. Deleted G-group chromosomes.



indeed be a result of the very short time interval between exposure to the drug and study of the cells. We also found, as did Cohen and co-workers,[2] substantial agreement between different slides from the same individual, different culture tubes, and repeat cultures from the same subject.

The most common type of abnormalities found were chromatid and isochromatid breaks. Although these are believed to occur in the periods before and after synthesis of deoxyribonucleic acid, after the usual culture time lymphocytes are often in the second or third division, and isochromatid breaks can appear as chromatid breaks either because of partial restitution or because an isochromatid break in the first division will appear in only one chromatid in subsequent divisions. Dicentric chromosomes and exchange figures were mainly seen in LSD users, but one of our controls showed three dicentric chromosomes, while another had one ring chromosome. In the user group, five subjects with a breakage rate under 10% had no dicentric chromosomes or exchange figures, whereas the 45 users with breakage rates above 10% showed 16 dicentric chromosomes and ten exchange figures. The increased number of breaks found in LSD users can explain, through occurrence of breaks in two chromosomes and subsequent healing, the presence of a large number of dicentric chromosomes. The situation is comparable to that induced in cultured cells by the simian virus 40 (SV 40).[8]

Table 2.—Chromosomal Damage In Adults Who Used LSD

Case No.	Sex	Total Dose (μg)	Interval Between Last Dose and Sampling (Days)	% Breaks	Other Drugs*
II-4	F	350	60	8.0	M
III-1	F	2,000	1	8.5	M, A
II-53	M	1,000	10	9.0	M
II-66	F	200	98	9.5	Ms
II-48	F	6,000	180	11.0	M, A, Ms, B, D, H, O
II-8	F	250	360	11.5	M
II-5	F	500	210	11.5	M
II-75	M	875	30	12.0	M
I-1	M	2,000	14	12.0	M
II-32	F	1,300	300	12.5	M, A, D
I-7	M	20,000	14	12.5	M
II-50	M	7,500	150	13.5	M, A, Ms, D, H
II-13	F	37,500	3	14.0	M, A
II-51	M	2,700	3	14.5	M, A
II-28	F	2,500	30	14.5	M
II-68	M	1,500	60	15.0	M, A
II-70	M	1,000	180	15.5	M, A
I-22	M	...	...	16.0	...
II-76	M	750	15	16.5	M
III-3	F	2,000	...	16.5	...
II-72	F	2,000	180	17.0	M, D, O
II-37	F	3,000	28	17.0	M
II-19	F	6,000	8	17.0	M
II-55	F	300	7	17.5	M, A
III-7	M	1,400	...	17.5	M
II-60	M	15,000	30	17.5	M, A, Ms, H, Mo
II-39	F	50,000	7	18.0	A, D
II-57	M	380	45	19.0	M, S
II-14	M	37,500	4	19.0	M, A
II-15	M	60,000	10	19.5	M, A
II-49	F	1,250	150	20.5	M
II-74	F	9,500	150	20.5	M, D
I-17	M	1,600	30	21.0	M
II-33	M	9,000	60	21.4†	M, A, Ms
II-42	M	4,500	24	22.0	M, A
II-12	F	9,000	30	22.0	M, A, Ms
II-3	M	3,500	60	22.5	M
II-21	M	12,500	3	23.0	M, A
I-10	M	15,000	180	23.0	M
I-15	M	36,000	90	25.0	M
I-20	M	25,000	3	26.0	M
I-13	M	1,000	...	28.0	...
I-5	M	10,000	21	31.5	M
I-9	M	70,000	1	38.0	M
II-24	M	10,000	14	41.5	M, A
I-19	M	12,000	1	45.0	M

*M, indicates marihuana; A, amphetamines; Ms, mescaline; B, barbiturates; D, dimethyltryptamine (DMT); H, heroin; O, opium; Mo, morphine; and S, 2, 5-dimethoxy-4-methyl-amphetamine (STP).
†Only 140 metaphases were studied in this patient.

Table 3.—Chromosomal Damage in Infants Exposed to LSD in Utero*

Case No.	Mother No.	Age of Infant, Mo	Sex	Doses in Utero			% Breaks
				No.	Amount (μg)	Time	
II-6	II-4	6	M	1	150	1st mo	9.5
II-40	II-48	18	M	6	250	1st 2 mo	22
II-43	II-45	1	M	5	150	2nd trimester	26.5
II-25	II-12	3	F	1	500	3rd mo	28
				2	500	5th mo	
				2	500	3rd trimester	

*All of these infants were also exposed to marihuana in utero.

A possible danger of such changes is an increase in the incidence of leukemia and other types of neoplasia in the large number of LSD users. It is well known that a chromosomal picture resembling that found in LSD users characterize ataxia telangiectasia, Bloom's syndrome, and Fanconi's anemia, which are autosomal recessive syndromes accompanied by an increased incidence of neoplasia and leukemia.[9-15] Similar chromosomal abnormalities are caused by a variety of carcinogens such as viruses and radiation; as a matter of fact, neoplastic cells practically always show a wide variety of chromosomal aberrations.

Long-lived chromosomal damage was evident in several of the patients in our study (up to 2 years following LSD ingestion), and in the group studied by Cohen et al[2] (up to 2½ years following ingestion). Long-lived chromosomal damage also has been demonstrated after therapeutic radiation[16]; this study emphasizes the relationship between chromosomal damage and an increased incidence of leukemia.

Another possible outcome of exposure to LSD is the production of congenital anomalies in the offspring. Stillbirths and stunting of development has been demonstrated in the offspring of rats injected with LSD on the fourth day of gestation[17]; gross brain and facial defects have been produced in the offspring of mice injected with LSD on the sixth

Table 4.—Distribution of Breaks Into Identifiable Chromosomes or Chromosome Groups

	A1	A2	A3	B	C	D	E	F	G	Total
Observed	129	105	45	93	322	58	27	3	9	791
Expected	71.82	66.83	55.84	100.29	274.31	83.41	72.29	38.36	27.76	790.9
X^2	45.52	21.8	2.1	0.52	8.29	7.74	28.37	32.59	12.67	159.6

df=8; $P < 0.001$

and seventh day of pregnancy.[18] Hamsters injected with LSD on the eighth day of gestation produced fetuses with a wide range of brain and spinal defects.[19] Recently Zellweger et al[7] described the case of a female child with a deformed right leg born to a mother who had taken LSD on the 25th day after her last menstrual period and three times between the 45th and 98th days of pregnancy. The second dose had thus been taken during a period critical for the production of leg deformities.[20] The malformation in this child was a unilateral fibular aplastic syndrome. Exposure to the drug early in pregnancy might be more detrimental to the embryo in terms of miscarriages or congenital anomalies.

Still unknown is the potential for damage inflicted by LSD on the chromosomes of the gametes. It is logical to suppose that LSD can reach the germinal cells freely (although in lower concentration) and perhaps induce chromosomal damage analogous to that demonstrated for streptonigrin,[21] which is teratogenic in rats[22] and causes chromosomal damage to circulating lymphocytes.[23,24] Structural rearrangements include balanced reciprocal translocation which, through segregation, can result in chromosomal imbalance to the gametes and give rise to congenital anomalies. The carrier and many of its offspring being clinically normal, the consequences of chromosomal imbalance may not be detected for generations. The possibility that LSD may damage the germinal cells is now being investigated in our laboratory.

The dangers arising from behavioral derangements are obvious in view of recent reports.[25-27] In our group, three subjects have had recurrences of the LSD effects for 9, 12, and 27 months, respectively; in every case, the effects continue to recur as of this date. A good review of the current status of LSD has been written by Hoffer.[28]

It is interesting that morning glory seeds (ololiuqui or *Rivea corymbosa*), also used as hallucinogens by some LSD users and by some devoted exclusively to the seed, contains, among other alkaloids, D-lysergic acid amide. This is a powerful hallucinogen with 10% of the activity of LSD.[28] The possibility that ingestion of the seeds can cause chromosomal damage is now being investigated.

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